

Art Unit : 1633 1652 *with*  
Examiner : Delia M. Ramirez, Ph.D.

4. I believe this to be true because, as is our standard practice, on February 24, 1995, the original library containing the 18GC clone was prepared. In approximately June, 1995, the library was screened for alpha-galactosidase activity, yielding 18GC as one of the clones with alpha-galactosidase activity. The clone was then isolated, labeled as "18GC1", and

one portion was stored in a glycerol stock at  $-80^{\circ}\text{C}$  and another portion was sent for sequencing. The clone was disclosed in U.S. Patent Application Serial No. 08/613,220, filed March 8, 1996.

From the frozen glycerol stock, a portion was recultured and the DNA isolated. This DNA was used for construction of an over-expression clone, also called a subclone. In this over-expression construct, the ORF (Open Reading Frame) was put under the control of an inducible promoter, the start codon was changed to 'ATG' and a C-terminal tag of six Histidine residues were added. The 18GC subclone was assigned a new name, 18GC1.11.1QET1. This subclone was prepared in a 15% glycerol stock, divided into 3 aliquots (1 ml aliquots in 1.5 ml vials), labeled as "GLY001-10, 18GC1.11.1QET1, 6/17/97" and stored at  $-80^{\circ}\text{C}$ . Two of the vials were sent off-site for storage and 1 vial remained at Diversa.

When it was time to prepare the 18GC subclone for ATCC deposit, I went to the person in charge of maintaining all of the frozen clones and subclones, requested vial containing the 18GC subclone. I thawed the 18GC sample and took a loop of it to start a culture. Once the 18GC subclone culture attained sufficient growth, I prepared 30 glycerol stocks of the culture and froze them for shipment to the ATCC for deposit. On September 9, 2002, Lynn Linkowski, of Diversa Corporation, coordinated and executed the deposit of the glycerol stocks of 18GC with the ATCC. The samples were received by ATCC on September 10, 2002, and assigned Patent Deposit Designation PTA-4654.

5. Accordingly, I submit that the deposited material, PTA-4654, was in our possession at the time of filing of the instant application, as well as the filing of the earliest priority application, and that the deposited material is the same material as that described in the specification as 18GC.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Applicant : Murphy, et al.  
Serial No. : 09/886,400  
Filed : June 20, 2001  
Page : 3

Attorney's Docket No.: 09010-004005

Respectfully Submitted

Date: 12/17/02

A handwritten signature in black ink, appearing to read 'Walter Callen', written over a horizontal line.

**Walter Callen**  
**Staff Scientist II**